

NUCLEIC ACID HYBRIDIZATION

DNA can be denatured and renatured. The process of strand separation is called denaturation (colloquially called melting). Heating or exposure to low salt concentrations destabilizes the noncovalent forces that stabilize the double helix, and this results in strand separation. DNA denaturation occurs over a narrow temperature range. For a particular DNA molecule, the melting temperature is influenced by the proportion of GC base pairs. The more GC base pairs, the higher the temperature necessary to denature the DNA.

If conditions are brought back to normal (e.g., physiological temperature), the single stranded (denatured) DNA can renature or reform complementary strands according to the rules of base pairing (A pairs with T and G pairs with C). When the renatured DNA strands are precisely complementary, the original double stranded helical structure can reform. These same biological features of DNA make it possible to manipulate nucleic acids in vitro.

When any two nucleic acids pair together by virtue of complementarity they are said to anneal with each other and form a duplex structure. When the nucleic acids are from different sources, as occurs when one preparation consists of DNA and the other RNA, the annealing process is described as hybridization. The two common ways of performing these reactions in vitro are solution hybridization and filter (or solid support) hybridization.

Nucleic acid hybridization - the formation of a duplex between two complementary sequences, usually between two molecules that have complementary bases. It is possible for a single strand of nucleic acid that has inverted repeat sequences to hybridize back onto itself forming a stem and loop structure.

DNA - DNA hybridization
DNA - RNA hybridization

Hybrid Stability

Intrinsic factors

A duplex with relatively more GC base pairs than AT base pairs will be more stable because there are three hydrogen bonds between G and C and only two between A and T
Thus, it would take a higher temperature to denature

A GC base pair-rich duplex
The degree of complementarity between two strands also influences stability.

Extrinsic factors (experimental conditions)

1. temperature
2. salt concentration
3. presence of denaturing agents (e.g., formamide)
4. presence of high molecular weight polymers (e.g., dextran sulfate)

Temperature

Ideal = 25 C below duplex melting temperature
High temperatures may damage nucleic acids

Salt concentration

Hybridization rate increases between 0.1 M and 1.2 M
Commonly use 5 to 6 x SCC for solid support hybridization
1 x SCC = 0.15M NaCl & 0.015M sodium citrate at
pH 7.2 to 7.4

During washing the amount of SCC is lowered depending upon required stringency

Denaturing agents

Every 1% formamide allows lowering temperature 0.7 C without losing specificity

Concentrations of 50% or greater formamide favor DNA-RNA hybridization over DNA-DNA hybridization

High molecular weight polymers

Effectively increase concentration of nucleic acids by excluding volume from the hybridization mixture

Stringency

By manipulating temperature and salt concentration, one can distinguish between perfect duplexes and duplexes that have mismatches between bases

Under stringent conditions only perfect or near perfect duplexes can be formed

The melting temperature of a duplex decreases 1 C for every mismatched base pair

Relaxed conditions that allow duplex formation with mismatched base pairs include lowering the temperature

Stability of duplexes with mismatched base pairs is favored by a higher salt concentration
Wash conditions on solid supports can be adjusted to achieve the desired amount of stringency
Under stringent conditions, wash temperature can be increased and salt concentration can be decreased (down to 0.1 x SCC)

Solid Support Hybridizations

Denatured DNA or RNA is immobilized on an inert support (filter hybridization)

- Prevents self-annealing

- Bound sequences available for hybridization with an added nucleic acid (the **probe**)

- Support filters

 - Nitrocellulose filters (most commonly used)

 - Nylon membranes (less brittle than nitrocellulose)

 - Cellulose paper impregnated with diazo groups

 - Diazo groups covalently bind to guanine residues on the DNA or RNA to stabilize support

Types of solid support hybridizations

- Dot/Slot blots**

- Southern (DNA) blots**

- Northern (RNA) blots**

Dot/Slot blots

- DNA or RNA is bound directly to the solid support filter and then hybridized to the probe

- Good for multiple samples and quantitative measurements

 - Specificity for qualitative measurements may be a problem for close but not identical sequences

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Specific Hybridization Applications

In situ Hybridization

FISH

Molecular Arrays

Hybridization Probes and Methodologies

Radioactive versus nonradioactive

Purified insert versus vector

Labeling methods

- Nick translation

T4 DNA polymerase
End-labeling with T4 polynucleotide kinase
End-labeling with terminal deoxynucleotidyl transferase
End-labeling with the Klenow fragment of E. coli DNA polymerase
Random primer
Polymerase chain reaction
Riboprobes
Removal of unincorporated label after probe preparation
Use of oligonucleotides
Denatured double-stranded DNA probes

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